Effects of Protect-It on Efficacy of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) Parasitizing Rice Weevils (Coleoptera: Curculionidae) in Wheat

J. PEREZ-MENDOZA, I. E. BAKER, F. H. ARTHUR, AND P. W. FLINN

Grain Marketing Production and Research Center, USDA-ARS, 1515 College Avenue, Manhattan, KS 66502

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ABSTRACT The parasitoid Anisopteromalus calandrae (Howard) was very sensitive to direct contact with Protect-It, an inert dust formulation containing 90% diatomaceous earth and 10% silica aerogel. LT_{50} s at room temperature and humidity were 49 min (95% CL = 48-51) and 72 min (95% CL = 69-74) for males and females, respectively, in petri dishes containing 2.5 mg dust per square centimeter. Under the same conditions, adults of the rice weevil, Sitophilus oryzae (L.) (Coleoptera: Curculionidae), a host of A. calandrae, were much less sensitive. Mortality of S. oryzae after a 24-h contact period was 50% for females and 62% for males. When tested in no-choice laboratory bioassays at 27°C, label rates of Protect-It (200 and 400 ppm) dusted onto hard red winter wheat, Triticum aestivum L. (13.9% moisture content) that was infested with immature rice weevils reduced the longevity of parent A. calandrae females and significantly reduced parasitization of the weevils at 3 tested humidities, 43, 60, and 75% RH. Parasitoid progeny production was also significantly reduced. At 75% RH, 92.8 \pm 2.9 parasitoid progeny were produced in untreated wheat compared with 12.6 \pm 2.6 progeny in the treated wheat. Sex ratio of parasitoid progeny was not significantly affected by the dust treatments at any relative humidity. In two-choice tests in divided petri dish arenas, single A. calandrae females showed a strong avoidance of Protect-It-treated wheat and a significant preference for parasitizing weevils in untreated wheat. However, significantly more parasitoid progeny were produced in dishes in which one-half contained Protect-It-treated wheat and the other half contained untreated wheat compared with dishes in which both halves of the divided dishes held untreated wheat. Reasons for the Protect-It-stimulated oviposition response by A. calandrae are not known, but may be related to stress induced by the dust. In separate tests, there was no significant difference in emergence of weevils from treated or untreated wheat, regardless of the weevil age at time of dusting, or the relative humidity at which the dusted wheat was maintained. Our studies provide evidence that any natural control of pest insects exerted by local populations of parasitoids, or enhanced biological control by augmentative releases of parasitoids, would be adversely affected by the use of Protect-It or other diatomaceous earth products on stored grain.

KEY WORDS inert dust, diatomaceous earth, parasitoid, biological control, stored products

AUGMENTATIVE RELEASE OF beneficial insects can be an effective management tool in agricultural ecosystems (Parrella et al. 1992), including the stored grain ecosytem (Brower et al. 1996, Schöller et al. 1997). One advantage that parasitoids and predators have is that releases into grain storages can be easily integrated with most recommended pest management protocols, including sanitation, aeration (Flinn 1998), and even certain grain protectants (Baker and Throne 1995). With the declining use of insecticides to protect grain during storage, alternatives such as diatomaceous earth and other inert dusts are being reformulated and reevaluated as insect control products (Golob 1997). It is not known how the increased use of diatomaceous

earth for pest management in the storage ecosystem may affect associated parasitoids and predators that provide biological control.

Efficacy of diatomaceous earth against stored grain insect pests is related to several physical properties of the material, including bulk density, effect on grain test weight, adherence to grain kernels, and $\mathrm{SiO_2}$ content (Korunic 1997). Insects treated with inert dusts undergo lethal desiccation (Alexander et al. 1944) thought to result from both abrasion of the cuticle surface and adsorption of lipid from the epicuticular lipid layer (Ebeling 1961, Ebeling and Wagner 1961. Baker et al. 1978) demonstrated selective adsorption of cuticular branched-chain alkanes from insects treated with finely powdered tricalcium phosphate. Because of the desiccating effect of inert dusts, grain moisture content affects efficacy of these compounds (Le Patourel 1986).

Sensitivity of a given insect species to inert dusts varies with formulation and source material (Korunic

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¹ Current address: Agustin Lanuza No. 118, Zona de Oro 1, Celaya, Guanajuato 38020, Mexico.

² To whom reprint requests should be sent.

1997). In addition, adults and larvae of different insect species can also vary in their sensitivity to the same formulation (Subramanyam et al. 1994, 1998). Among the complex of parasitoids associated with stored grain (Brower 1990), some would be considered soft-bodied and some are more heavily sclerotized, which may affect their response to diatomaceous earth. Also, a number of species penetrate grain masses during host searching and others are located primarily on the grain surface. As a result, searching behavior among these species could affect the degree of their exposure to inert dusts. Here we report results of direct contact studies, as well as results of no-choice, and 2-choice bioassays with Protect-It, a formulation containing 90% diatomaceous earth and 10% silica aerogel (Korunic and Fields 1995), on Anisopteromalus calandrae (Howard) parasitizing rice weevils, Sitophilus oryzae (L.), in hard, red winter wheat. A. calandrae is a species that easily penetrates grain masses during host searching where it would come into extensive direct contact with dusts used as grain protectants. Tests were conducted with 2 dose levels of Protect-It and with 3 relative humidities.

Materials and Methods

Insects. Cultures of S. cryzae were maintained on hard, red winter wheat (moisture content of 13.9%) at 27°C and 60% RH. A malathion-susceptible laboratory strain of A. calandrae maintained on cultures of S. cryzae was used in this study. Adult parasitoids that had been emerged for 2–3 d were aspirated from stock cultures, briefly anesthetized with CO_2 to facilitate handling, and placed in the different treatment arenas with a soft brush.

Direct Contact Bioassay. Direct contact bioassays were used to compare responses of male and female A. calandrae and adults of S. oryzae to a single dose of Protect-It (Hedley Technologies, Mississauga, ON). Anisopteromalus calandrae were briefly anesthetized and placed in plastic petri dishes (100 by 15 mm) containing 31 mg of Protect-It (also referred to below as diatomaceous earth) (2.5 mg/cm²). Mortality of A. calandrae was determined at 10-min intervals. Results are based on 2 tests at room temperature, with 18 adults per replicate, and 5 replicates per test. Control dishes without diatomaceous earth were included for each sex. Mortality data were analyzed by the probit procedure for correlated data by Throne et al. (1995a). Probability of dying was obtained from back transformations (Throne et al. 1995b) of the transformation giving the best fit to the data as determined by chi-square goodness-of-fit.

Effect of Protect-It on mortality of male and female adults of *S. oryzae* (5 replicates per sex, 10 weevils per replicate) was determined after 24 h at room temperature.

No-Choice Bioassay. Effect of 2 doses of diatomaceous earth tested at 3 humidities on efficacy of *A. calandrae* parasitizing rice weevils at 27°C in hard red winter wheat was determined as follows. Rice weevil hosts were prepared by placing 400 adults (1–2 wk

old) of S. oryzae in each of two 3.7-liter jars containing 1 kg hard winter wheat (13.9% moisture content). After 7 d the weevils were removed by screening. After 21 d the wheat from the 2 jars was combined, thoroughly mixed, and 20-g samples were weighed and placed into each of 120 plastic vials (3.2 by 8 cm) with snap-cap lids containing 40-mesh screens. Appropriate amounts of diatomaceous earth, equivalent to 200 or 400 ppm on wheat, were weighed and placed in the vials. Each dose was tested at each of 3 humidities maintained with saturated solutions of K₂CO₃ (43% RH), NaBr (60% RH), and NaCl (75% RH). The saturated solutions were placed in the bottoms of clear, plastic boxes (15 by 27 by 38 cm) that had an elevated plastic grid to support the vials. For each dose/humidity combination the following vials were prepared: 5 vials with diatomaceous earth and with 5 female A. calandrae; 5 vials without diatomaceous earth and with 5 female A. calandrae; 5 vials with diatomaceous earth and without A. calandrae; and 5 vials without diatomaceous earth and without A. calandrae. After 5 d the parasitoid females were removed from each vial and mortality was noted. After an additional 21 d, all vials were placed in the freezer and emerged male and female parasitoid progeny as well as emerged weevils for all treatment combinations were counted.

Data were analyzed by analysis of variance (ANOVA) (SAS Institute 1987). Experimental design was a split-plot with emerging weevils, parasitoid progeny, and humidity levels arranged in a 2×2 factorial as the main plot and with diatomaceous earth dose at the subplot level. Means were compared by using a least significant difference (LSD) test (Steel and Torrie 1980).

Two-Choice Bioassays. In this test, single A. calandrae females were placed in a divided petri dish and given the choice of parasitizing 21-d-old weevils in untreated wheat or diatomaceous earth-treated wheat. In addition, the possibility that choice might be affected by host availability was tested with a time component (i.e., females were removed after a 24-, 48-, 72-, or 96-h exposure to the weevil-infested wheat in the dishes). In these tests a single diatomaceous earth dose (400 ppm) was tested at 60% RH. Details are as follows: wheat containing 21-d-old weevil hosts was obtained as above. Samples of untreated (10 g) and diatomaceous earth-treated wheat (10 g) were placed in opposing sides of a divided plastic petri dish (15 by 100 mm). Sixty-five petri dishes were prepared. A single female A. calandrae was added to each dish (except for control dishes used to monitor weevil emergence). After 24, 48, 72, and 96 h, 10 replicate dishes were removed and the wheat on each side of the divider was separately transferred to petri dishes (15 by 60 mm). After 21 d, all samples were frozen and emerged male and female parasitoids, as well as rice weevils, were counted.

Data were analyzed by using ANOVA with a factorial design (SAS Institute 1987) with time as the main plot and dose as the subplot. Means were compared by using the LSD test (Steel and Torrie 1980).

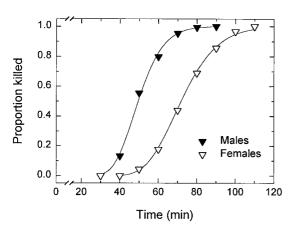


Fig. 1. Time-mortality curves for male (solid triangles) and female (open triangles) A. calandrae exposed to Protect-It (2.5 mg/cm2) in petri dishes at room temperature. Points are means \pm SE based on direct contact mortality data from 2 tests with 5 replicates, 18 adults per replicate per sex per test. Lines are back transformations from log-probit analyses of the data.

Effect of Diatomaceous Earth on Emergence of Weevils from Treated Wheat. Tests were conducted to determine if Protect-It would affect the emergence of weevils from treated wheat. For these studies, 1 dose of diatomaceous earth was used (400 ppm). Weevils (400 adults) were allowed to oviposit in 1 kg wheat for 7 d. Samples from a common batch of wheat were isolated after 7, 14, 21, and 28 d. At each time, 30 samples (each 20 g) were weighed into plastic vials (2.5 by 8 cm) with screen lids. Diatomaceous earth was weighed into 15 of the vials. Five vials containing untreated wheat and 5 vials with diatomaceous earthtreated wheat were placed into containers of saturated salt solutions maintaining 42, 60, or 75% RH. After 35 d at 27°C, all vials were frozen and weevils that had emerged were counted.

Emergence data were analyzed with ANOVA (SAS Institute 1987).

Results

Direct Contact Bioassays. Adult A. calandrae were very sensitive to Protect-It when this formulation was used as a direct contact inert dust (Fig. 1). The LT $_{50}$ for males was 50 min (95% CL = 48–51) at room temperature. Females were somewhat less sensitive than males with an LT $_{50}$ of 72 min (95% CL = 69–74). Complete mortality of males occurred in <90 min. All female wasps died in <120 min. When adults were first placed in the treated dishes, diatomaceous earth particles adhered tightly to the cuticle surface and the wasps were observed to groom extensively, apparently attempting to remove the particles.

Compared with the parasitoids, the adult weevils were much less sensitive to direct contact with Protect-It. After 24 h, there was 50% mortality among the females and 62% mortality among the males.

No-Choice Bioassays. After the 5-d oviposition period, there was high mortality of the parent A. calandrae screened from the vials used in the no-choice bioassay. At 200 ppm, mortality was 76, 76, and 60% at 43, 60, and 75% RH, respectively. At 400 ppm, mortality was 96, 100, and 96% at 43, 60, and 75% RH, respectively. Control mortality was 6, 10, and 4% at 43, 60, and 75% RH, respectively. In addition to reducing the life span of the parent female wasps, Protect-It dusted onto wheat that was infested with immature rice weevils significantly affected the efficacy of A. calandrae during the parasitization of these hosts (Table 1). Across all treatment combinations, the total number of wasp progeny produced was affected by both relative humidity (F = 18.46; df = 2, 72; P = < 0.01) and diatomaceous earth dose (F = 560.43; df = 2, 72; P =< 0.01). More total parasitoid progeny were produced at higher humidities, whereas the number of parasitoid progeny was dramatically reduced as the diatomaceous earth concentration increased. Sex ratio of emerging parasitoid progeny was not affected by relative humidity or dose of diatomaceous earth (F =0.13; df = 1, 72; P = 0.719). There was a weak but significant interaction at the 0.05 level between relative humidity and diatomaceous earth dose on the production of parasitoid progeny (F = 3.48; df = 4, 72; P = 0.012), primarily because of reduced numbers of progeny in controls at low RH.

At a given humidity, total parasitoid progeny, number of female and male parasitoids emerging, and percentage of parasitization decreased significantly with diatomaceous earth dose. However, because the diatomaceous earth adversely affected the parasitoids, the number of weevils emerging from the wheat increased as the diatomaceous earth dose was increased.

Two-Choice Bioassays. In the two-choice divided petri dish arenas, single female A. calandrae avoided the diatomaceous earth-treated wheat. Although we did not study any behavioral responses to the diatomaceous earth -treated grain per se, the parasitoids showed a strong preference for ovipositing on hosts in untreated wheat, as opposed to ovipositing on hosts in wheat dusted with Protect-It (F = 266.43; df = 1, 72; $P = \langle 0.01 \rangle$ (Fig. 2). During the first 72 h, there was little parasitization of rice weevils in diatomaceous earth-treated wheat (Fig. 2A). For example, the mean number (±SD) of parasitoid progeny emerging from untreated wheat after a 72-h oviposition period was 18.7 ± 8.2 compared with 2.7 ± 2.1 on the diatomaceous earth-treated wheat. Between 72 and 96 h, avoidance of the diatomaceous earth-treated wheat was somewhat lessened. The mean number of progeny emerging from the diatomaceous earth-treated wheat did increase to 9.4 ± 4.1 compared with 31.9 ± 3.2 from untreated wheat. The increased utilization of hosts in the diatomaceous earth-treated wheat may result from the reduction in available hosts on the untreated side.

Because the parasitoids avoided the diatomaceous earth-treated wheat, there were significantly more weevils emerging from the diatomaceous earth-treated wheat compared with untreated wheat in the same dishes (Fig. 2B) (F = 10.95; df = 1, 72; P =

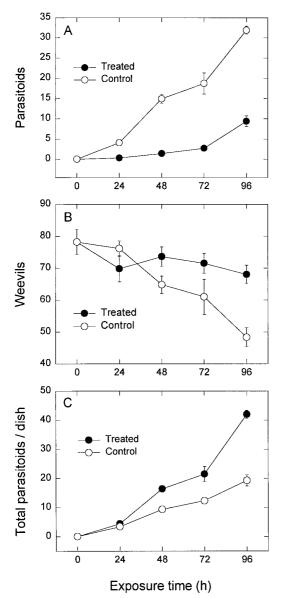


Fig. 2. Results of 2-choice bioassays in which Protect-It-treated wheat (400 ppm) containing weevil hosts for *A. calandrae* was placed on one side and untreated wheat was placed on the opposite side of a divided petri dish. (A) Number of parasitoid progeny produced in untreated wheat (open circles) and diatomaceous earth-treated wheat (solid circles) as a function of foraging time by the parent female parasitoid. (B) Number of weevils emerging from untreated wheat (open circles) and diatomaceous earth-treated wheat (solid circles) as a function of exposure time to the parent *A. calandrae* female. (C) Increase in the number of parasitoids produced from dishes in which diatomaceous earth was present (solid circles) compared with dishes in which no diatomaceous earth was present (open circles).

< 0.01). Similarly, during the 96-h oviposition period for each *A. calandrae* female, the percentage of parasitization of host weevils (based on number of wee-

vils emerging from unparasitized controls) was significantly reduced in the diatomaceous earth-treated wheat compared with that in untreated wheat (F = 10.94; df = 1, 72; P = < 0.01).

When compared with the total number of parasitoid progeny that emerged from control dishes where both halves of the dish contained untreated wheat, there were significantly more parasitoid progeny produced in dishes in which one-half had diatomaceous earth-treated wheat and one-half had untreated wheat (Fig. 2C) (F = 111.95, df = 1, P = < 0.01). Although female A. calandrae parasitized weevils that were almost exclusively in untreated wheat in dishes in which diatomaceous earth was present, the presence of diatomaceous earth in these dishes apparently increased their rate of parasitization of the hosts. The mechanism by which diatomaceous earth exerted this stimulatory effect is unknown.

Effect of Diatomaceous Earth on Development and Emergence of Rice Weevils in Treated Wheat. The number of weevils emerging from diatomaceous earthtreated wheat was not significantly different from those emerging from untreated wheat (F = 0.18; df = 1, 96; P =0.67), regardless of the larval age (F = 2.27; df = 3, 96; P =0.085) at which the diatomaceous earth was dusted onto the wheat, or the relative humidity (F = 1.99; df = 2, 96; P = 0.143) at which the wheat was maintained. Immature weevils in wheat that was dusted 7 d after oviposition would be exposed to any adverse effects of the diatomaceous earth for the longest time period (i.e., from 1st instar to adult). Under these conditions, the mean number of weevils emerging from wheat that was dusted at 7 d and held at 75% RH was 203.6 \pm 11.5 compared with 191.8 ± 11.9 weevils in untreated wheat (F = 2.55; df = 1, 8; P = 0.149).

Discussion

Depending on formulation, inert dusts can provide efficaceous control of many stored grain insect pests. Protect-It is effective against the rusty grain beetle, Cryptolestes ferrugineous (Stephens), and the red flour beetle, Tribolium castaneum (Herbst) (Korunic et al. 1996) and is labeled for use as a protectant in the United States against most primary and secondary insect pests of stored grain. This inert dust formulation can synergize heat treatments and may be useful in commercial scale heat treatments to control insects in food processing plants (Fields et al. 1997), but our results indicate that the successful integration of inert dusts with biological control technologies, such as parasitoids, may be difficult. Adults of A. calandrae were very sensitive to direct contact by the dust, and their longevity and effectiveness in parasitizing immature weevils was significantly reduced by the label rate of Protect-It dusted onto wheat. Nevertheless, our laboratory results do indicate that the inkernel-feeding hosts for A. calandrae are not affected by the dusts applied to the grain and that adult parasitoids can avoid treated grain. As a result, it may be possible that some biological control can occur in stored grain even in the presence of inert dust treatments. In addition,

Table 1. Effect of Protect-It dusted onto hard red winter wheat infested with rice weevil, S. oryzae, on parasitization by the pteromalid wasp A. calandrae

	No. of wasps or weevils emerged (mean ± SEM) per replicate					
${ m DE} \over { m dose}^a$	Total A. calandre progeny	A. calandrae females	A. calandrae males	A. calandrae sex ratio ^b	Total weevil emergence	$\%$ parasitization c
	43% RH					
0	$79.0 \pm 2.9a$	$37.2 \pm 2.6a$	$41.1 \pm 2.2a$	$0.9 \pm 0.1a$	$44.8 \pm 2.7a$	$72.7 \pm 1.7a$
200	$35.0 \pm 3.0b$	$15.0 \pm 1.1b$	$20.0 \pm 2.0b$	$0.8 \pm 0.0a$	$80.6 \pm 4.2b$	$31.6 \pm 3.6b$
400	$10.2\pm1.6c$	$5.0 \pm 1.2c$	$5.2 \pm 0.7c$	$0.9 \pm 0.2a$	$125.2 \pm 3.7e$	$10.8\pm2.6c$
			60%	RH		
0	$84.2 \pm 4.0a$	$43.3 \pm 3.6a$	$40.6 \pm 2.3a$	$1.1 \pm 0.1a$	$47.6 \pm 1.0a$	$67.6 \pm 0.7a$
200	$38.6 \pm 1.9b$	$18.6 \pm 1.7b$	20.0 ± 0.7 b	$0.9 \pm 0.1a$	$83.2 \pm 5.6b$	$36.5 \pm 4.2b$
400	$7.6 \pm 1.4c$	$3.4 \pm 0.7c$	$4.2 \pm 0.9c$	$0.9 \pm 0.2a$	$135.4 \pm 4.7c$	$6.7 \pm 3.2c$
			75%	RH		
0	$92.8 \pm 2.9a$	$47.8 \pm 2.6a$	$44.8 \pm 0.9a$	$1.1 \pm 0.1a$	$35.4 \pm 2.8a$	$76.3 \pm 1.9a$
200	$54.8 \pm 4.8b$	$30.0 \pm 2.9b$	$26.8 \pm 2.5b$	$1.1 \pm 0.1a$	$72.0 \pm 3.8b$	$48.6 \pm 2.7 b$
400	$12.6 \pm 2.6c$	$5.8 \pm 2.1c$	$6.8 \pm 0.9c$	$0.8 \pm 0.2a$	$136.2 \pm 3.3c$	$3.9 \pm 2.3c$

appm on wheat

release technologies perhaps can be developed that can minimize the effects of diatomaceous earth on parasitoids. For example, top dressing of grain with diatomaceous earth is a common practice. Releasing parasitoids in the bottom of grain bins or dispensing parasitoid pupae within grain kernels throughout the grain mass during bin loading may be alternative parasitoid release mechanisms that might be compatible with top dressing of grain with diatomaceous earth.

The mechanism by which Protect-It stimulated an increase in parasitization by *A. calandrae* in dishes that contained the diatomaceous earth is not known. Although half of the wheat in the dishes was not treated with the dust, it is likely that the parasitoids came into contact with a sufficient number of dust particles to result in mild dehydration. It is known that stress can cause fluctuations in hormone levels in insects (Cook and Holman 1985), and the stress induced by dehydration may be the stimulus for increased foraging and parasitization in *A. calandrae*.

In studies initiated to explain why outbreaks of scale insects often occurred along dusty roadways in citrus orchards, 2 species of parasitic Hymenoptera associated with citrus, Aphytis chrysomphali (Mercet), a relatively soft-bodied aphelinid, and Metaphycus luteolus (Timberlake), a more sclerotized encyrtid parasitoid, were found to be very sensitive to a range of mineral-based inert dusts (Bartlett 1951). Based on LT₅₀s, A. chrysomphali was much more sensitive to dusts than was M. luteolus. Dusts adhered much more strongly to the soft-bodied wasp. In the direct contact studies with A. calandrae, we also observed strong adherance of the diatomaceous earth-silica aerogel formulation to the wasp cuticle. Anisopteromalus calandrae is a small, robust wasp that would be considered heavily sclerotized. In preliminary direct contact studies, Protect-It also completely covered the cuticle of a more soft bodied parasitoid found in the stored grain ecosystem, Habrobracon (Bracon) hebetor Say

(unpublished data). In contrast to A. calandrae, H. bebetor does not penetrate deeply into grain masses but prefers to forage on the grain surface. Despite these differences in behavior and body structure, both parasitoids were very sensitive to Protect-It.

Based on LD₅₀s, the toxicity of organophosphate grain protectants against the laboratory strain of A. calandrae was chlorpyrifos-methyl > pirimiphos methyl > malathion (Baker and Weaver 1993). Results from glass vial bioassays of parasitoid and host indicated that A. calandrae was \approx 21-fold more sensitive to chlorpyrifos-methyl and \approx 8-fold more sensitive to pirimiphos-methyl and malathion than was its host S. oryzae. Based on LT₅₀s in the current study, Protect-It is \approx 20- to 25-fold more toxic to A. calandrae than to S. oryzae, and therefore the relative toxicity between parasitoid and host of this inert dust formulation is similar to that of chlorpyrifos-methyl.

Like many insecticides, effective formulations of inert dusts are nondiscriminatory in their action against both pest and beneficial insects. If inert dusts alter the lipid epicuticle of susceptible insects, more detailed studies on the mode of action of these materials may be necessary to allow development of formulations that would specifically target pest insects but would be less detrimental to beneficial species. Alternatively, there is some evidence that stored grain insects can develop tolerance or resistance to diatomaceous earth (Korunic 1998). Development of strains of parasitoids or predators that are resistant or less sensitive to diatomaceous earth would facilitate integration of these biological control agents with inert dust treatments.

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 $^{^{}b}$ Sex ratio based on number of female \div number of male parasitoids emerged.

^c Percentage of parasitization based on number of weevils emerged in separate controls without parasitoids for each relative humidity/dose combination.

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